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CONTRIBUTIONS TO THE BIOSYSTEMATICS IN THE SUBTRIBE *LIPARIDINAE* BENTH. (ORCHIDACEAE) WITH PARTICULAR REFERENCE TO SOUTH INDIAN SPECIES

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INTRODUCTION

The subtribe *Liparidinae* Benth. of the family Orchidaceae is characterised by the presence of naked pollinia. Lindley (1830) included this under the tribe Malaxideae and described four genera, viz. *Oberonia, Microstylis* and *Liparis* under the section Pleurothalleae. Hcoker (1888) treating Malaxeae as a subtribe of Epidendreae described 7 genera including *Oberonia, Microstylis* and *Liparis* under it. However, no separate genus *Malaxis* has been described by him. Until the publication of "A Revision of the genera *Microstylis* and *Malaxis*" by Ridley (1888) the genera *Oberonia, Malaxis, Microstylis* and *Liparis* as separate taxonomic entities were in a state of confusion. Even certain species of *Oberonia* for instance *O. ensiformis* Lindl., *O. verticillate* Wight and *O. brunoniana* Wight were included in the genus *Malaxis* by Reichenbach (1873) and Smith (1914). Later, Schlechter (1927) considered *Microstylis* Nutt., as a synonym to *Malaxis* Sw. Yet, in most of the South Indian Floras (Cooke, 1908, Fischer in Gamble, 1928 and Fyson 1932) the species of *Malaxis* have been described as *Microstylis* only. However, Santapau and Kapadia (1966) have considered species of *Microstylis* as belonging to *Malaxis.*

Such nomenclatural confusions are also seen existing within the genus. For instance, in case of *Oberonia, O. santapaui* Kapadia was earlier described as *O. lindleyana* Wight. But, the same species was also described as *O. lindleyi* and *Malaxis lindleyana* by other workers like Fischer (1861) and Kuntze (1892). Although, Santapau and Kapadia (1966) in their critical works on Bombay Orchids evolved a new name, *O. santapaui,* their observations were based upon Wight's earlier description. Under such circumstances a thorough study of fresh plant materials was felt necessary. In view of these confusions, a biosystematic study comprising morphological, cytological and biochemical data on the following 12 species of the genera *Oberonia, Malaxis* and *Liparis* occurring in Peninsular India was undertaken to delimit the species and to understand their phylogenetic relationship : 1. *Oberonia falconeri* Hook. f., 2. *O. verticillata* Wight, 3. *O. brunoniana* Wight, 4. *O. ensiformis* Lindl., 5. *O. santapaui* Kapadia, 6. *O. iridifolia* var. *denticulata* Wight, 7. *O. brachyphylla* Blatt. & McCann, 8. *O. proudlockii* King and Prantl., 9. *Malaxis versicolor* Sant. & Kapadia, 10. *M. densiflora* O. Kuntze, 11. *Liparis nervosa* Lindl. and 12. *L. prazeri* King & Pantl.

MATERIALS AND METHODS

Materials for the present study were obtained from various parts of Peninsular India. However, intensive collections were

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his paper is based on the data from the
is by the author.

made in Western Ghat regions from Castlerock in the North to Sakleshpur in the South. They were grown in the University Orchidarium at Dharwar. Each sample was analysed for morphological, cytological and biochemical characters. Biochemically free amino acids and flavonoids have been analysed by thin layer chromatography. The coloured spots obtained were recorded for their Rf-values and compared with the standard.

Cytological study was done as described in an earlier publication (Jorapur & Hegde, 1975).

Biochemically, flavonoids were analysed through 1-dimensional chromatography (Hegde & Jorapur, 1975). Free amino acids were analysed following Stahl's (1919) chromatographic technique. The plates were loaded with the standard amino acids of known Rf-values along with the extract, on the same abscissa. The coloured spots obtained were recorded for their Rf-values and compared with the Rf-values of the standard. The results were recorded (table 1 to 3).

Voucher specimens were deposited at the Herbarium of the Botany Department, Karnatak University, Dharwar.

RESULTS

All the 8 species in the genus *Oberonia* have equitant, flat and fleshy leaves and are epiphytic. Careful observations show that all the morphological features, especially of the lip vary from species to species consistently.

The genera *Malaxis* and *Liparis* are mostly terrestrial and pseudobulbous. The species of the genus *Malaxis* are distinct in having superior lip and very short and wingless column. The species of the genus *Liparis* possess inferior clawed lip without auricles and long and winged column.

Chromosome numbers for the genus *Oberonia* are $2n = 30$, whereas, in *Malaxis* and *Liparis* they are $2n = 42$.

Biochemical patterns for free amino acids and flavonoids are found to be very impressive (table 1 to 3). Free amino acids like dl-isoleucine and glycine are although found in almost all species there are certain "uncommon" amino acids specific to the individual species. Totally 9 free amino acids could be identified on the chromatogram. They are : dl-alanini (ALA), dl-aspartic acid (ASP), dl-isoleucine (ILEU), dl-ornithine monohydrochloride (ORN), dl-serene (SER), l-arginine (ARG), glycine (GLY), l-lysine monohydrochloride (LYS) and dl-threonine (THR). There are other coloured spots which could not be identified.

The flavonoids or "secondary substances" analysed under long wave UV light after spraying with aluminium chloride solution show three major fluorescent spot (marker) patterns each representing a genus. They are : I. *Oberonia* pattern, II. *Malaxis* pattern and III. *Liparis* pattern.

The *Oberonia* pattern is characterised by the greenish yellow (GY) and bluish yellow (BY) dominant fluorescent spots; *Malaxis* pattern with blue (B) and greenish yellow (GY) fluorescent spots and that of the *Liparis* pattern with blue (B) greenish yellow (GY) and red (R) dominant fluorescent spots. There are other minor fluorescent spots also which are less intensive. They are : Yellow (Y), yellowish blue (YB), yellowish brown (YBr) and reddish yellow (RY).

DISCUSSION

Biosystematics in the modern perspective is the biological characterisation and determination of the status of the species objectively. It is the synthesis of characters obtained from various disciplines like cytology, taxonomy biochemistry etc., to evolve an accep classification. (Davis and Heywood 1963, Harborne 1971, Heslop-Harrison, 1963). In the present study, these three aspects of approaches, viz. cytology, morphology and biochemistry supplement each other in establishing the individuality and elucidating the phylogenetic relationship among and between the species of the genera *Oberonia, Malaxis* and *Liparis.*

Phylogeny :

In the species of the genus *Oberonia* phylogenetic relationships could be envisaged in the lip characters—especially in the shape and articulation. The evolution in this case appears to be towards modification and specialization. As Dressler and Dodson (1959) have suggested, the simple lip is more like a petal. Considering the lip as the character for speciation, the simple lip as seen in *O. falconeri* might have given rise to a much modified and specialized lip seen in *O. proudlockii* (fig. 1).

Diploid chromosome number in the species of the genus *Oberonia* is found to be 2n =30. Earlier report (Chardard 1963, Tanka, 1965) on two species is also 2n = 30 in the genus *Oberonia.* On further analysis of the karyotypes, it has been possible to know the individuality of the species on the one hand, and evolutionary status of each species on the other. From the cytological data it appears that the karyotype evolution in the species of *Oberonia* is towards—

i) gradual reduction in the total chromatin length;

ii) gradual reduction in the size of long chromosomes;

iii) gradual increase in the number of short chromosomes; and

iv) structural changes of the chromosome.

This trend of evolution of chromosomes goes hand-in-hand with the lip modification and specialization in the case of *Oberonia* (Jorpur and Hegde, 1975). In the evolutionary line-up it appears that *O. falconeri* with its simple karyotype, long chromosome (4 μ and above and more total chromatin length (49.33 μ) is primitive and *O. proudlockii* with its more numerous short chromosomes (2.50 μ and below) and least chromatin length (27.79 μ) is advanced from among the species of *Oberonia* studied here. The sequence of phylogenetic relationship finds well correlated with the lip specialization (fig. 1) *O. brunoniana* on the other hand, deviates from *O. verticillata* not only in its lip character, but also in its cytological features, in comparison with *O. ensiformis* and *O. santapaui.*

The biochemical characters further substantiate the above view. As for the flavonoids (table 1), the derivation of relationship has been discussed elsewhere before on the genera *Oberonia, Malaxis* and *Liparis* (Hegde and Jorpur 1975). It has been shown also that biochemical characters are genetically controlled (Herney and Grant, 1964; Belzer and Ownbey, 1971 and Hegde and Jorpur, 1975).

From the data of Rf-values of free amino acids occurring in the genus *Oberonia,* it can be noted that dl-isoleucine is present in all the species studied. However, there are other free amino acids which are found only in the related species (table 3). For instance (i) in *I. verticillata, O. brunoniana* and *O. santapaui* the presence of two pink (P) spots with Rf-values (=0.21) and (=0.45) in all the three species; (ii) the occurrence of amino acids with Rf-values (=0.09) and (=0.24) in *O. ensiformis* and *O. santapaui*; (iii) the fact that both *O. santapaui* and *O. iridifolia* var. *denticulata* possess dl-ornithine monohydrochloride and glycine and (iv) the appearance of bluish pink (BP) spots with Rf-value (0.24) in all three species viz. *O. iridifolia* var. *denticulata, O. brachyphylla* and *O. proudlockii* : are probably indicative of their close relationship. At the same time it is interesting to note that there occur certain "uncommon" amino acids which are specific to the individual species. For instance *O. brunoniana* is characterised by the presence of dl-aspartic acid and another amino acid spot with blue coloration (=0.10) which are not found in other species of *Oberonia*. This fact supports the cytotaxonomical findings where *O. brunoniana* has deviated from the line of evolution.

Thus, in the genus *Oberonia* the biochemical characters have supplemented in justifying the cytotaxonomical findings (fig. 1).

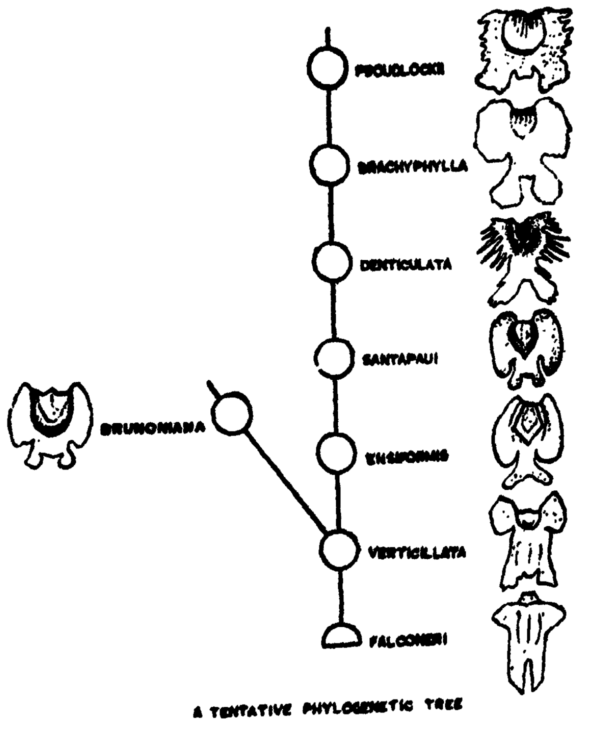

Fig. 1. : Phylogenetic trend with respect to lip characters in the genus *Oberonia*.

The species of the genus *Malaxis* show the somatic chromosome number to be $2n = 42$. However, there are karyotypic variations to distinguish the individual species.

The species in the genus *Liparis* also possess $2n = 42$ chromosome numbers. However, the karyotype is larger as compared to the karyotypes of the species of *Malaxis*.

Biochemically, the flavonoid and free amino acids provide additional characters to evaluate the individual species. The species of *Malaxis* show similarities in possessing glycine, dl-isoleucine and another uncommon amino acid spot (= 0.4). Yet, variation with respect to other spots justify their individuality (table 2 & 3).

Both the genera *Malaxis* and *Liparis* show certain similarities in possessing blue (B) and greenish yellow (GY) flavonoid marker spots. However, species of *Liparis* are distinctive in possessing red (R) fluorescent spots. Individual species of the genus *Liparis* also display specific patterns both for flavonoids and free amino acids (table 2 & 3).

Thus, from the above correlative study of morphological, cytological and biochemical characters, it is evident that in the subtribe Liparidinae, the three genera, *Oberonia*, *Malaxis* and *Liparis* are distinct entities. It appears probable that the genus *Oberonia* has diverged from *Malaxis* and *Liparis* long back in its evolutionary history. However, the later two genera, *Malaxis* and *Liparis* are closely related (fig. 2).

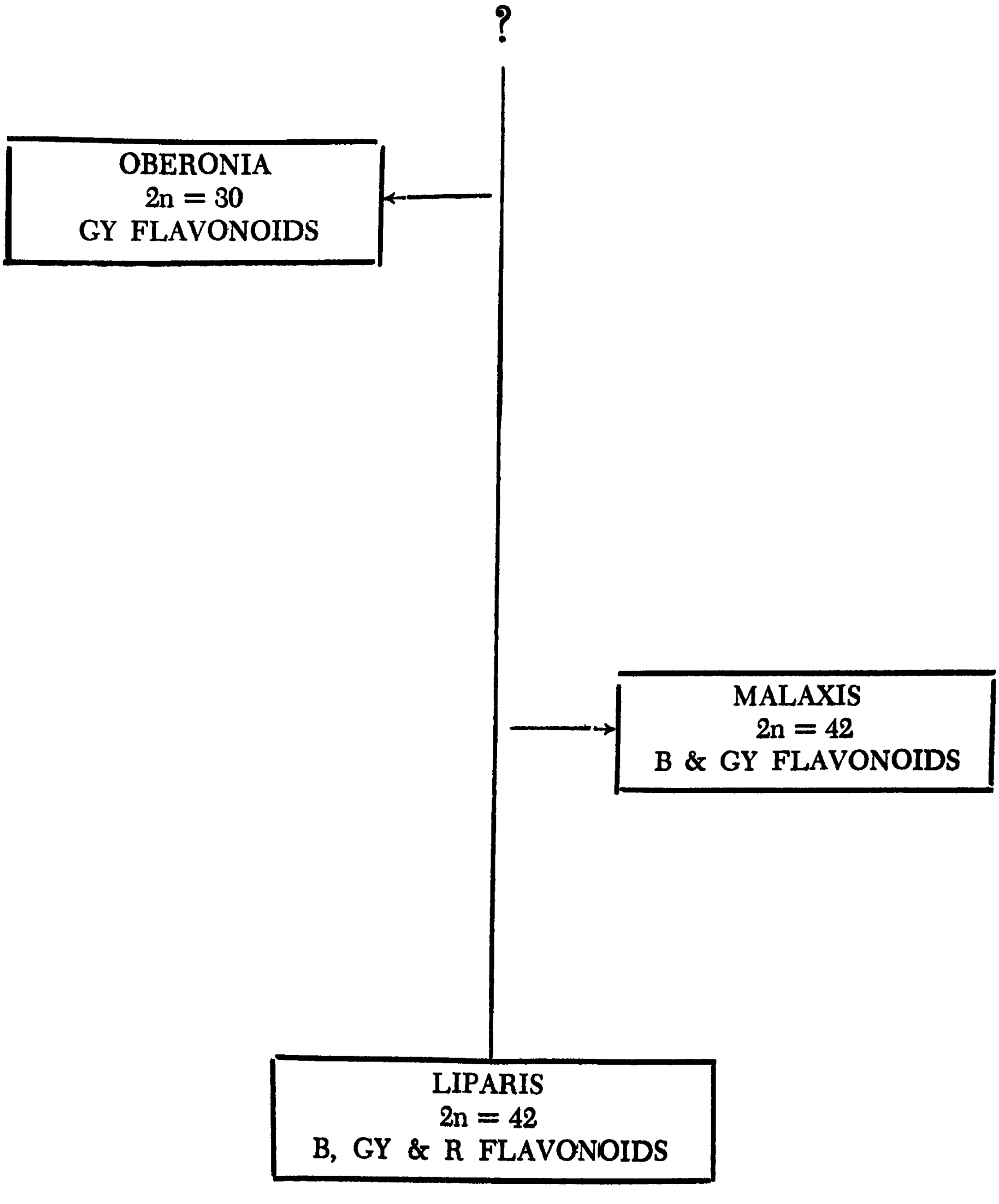

Fig. 2. : Tentative phylogenetic tree in the subtribe *Liparidinae*.

Table I : Flavonoid spot patterns in *Oberonia* Sw.

Sl. No.	Rf Value	Colour of spots in *Oberonia* pattern							
		1	2	3	4	5	6	7	8
1.	0·022	Y	Y	Y	—	Y*	—	—	—
2.	0·033	—	—	—	Y	—	—	—	—
3.	0·089	—	—	—	—	—	Y	Y	—
4.	0·14	Y	BrY*	—	Y	—	—	—	—
5.	0·15	—	—	Y	—	Y	—	—	—
6.	0·19	—	—	—	—	Y	—	—	—
7.	0·21	—	—	Y	GY*	—	—	—	—
8.	0·28	—	—	—	—	GY*	—	—	—
9.	0·30	—	—	—	—	—	GY*	—	—
10.	0·34	—	—	GY*	—	—	—	—	—
11.	0·36	—	—	—	—	—	—	—	Y
12.	0·37	—	GY*	—	—	—	—	—	—
13.	0·38	—	—	—	GY*	GY*	—	GY*	—
14.	0·42	Y	—	—	—	—	—	—	—
15.	0·48	—	—	—	—	—	Y	—	—
16.	0·50	—	—	GY*	—	—	—	—	—
17.	0·51	—	—	—	—	—	—	GY*	—
18.	0·54	Y	—	—	—	—	—	—	—
19.	0·60	—	GY*	—	—	—	—	—	—
20.	0·63	—	—	—	—	—	—	—	Y
21.	0·65	—	—	—	—	—	GY*	GY*	—
22.	0·68	YB	—	—	—	—	—	—	—
23.	0·62	—	—	GY*	GY	GY	—	—	—
24.	0·70	GY*	—	—	—	—	—	—	—
25.	0·72	—	—	—	—	—	—	—	GY*
26.	0·77	—	Y	—	Y	Y	—	—	—
27.	0·78	—	—	—	—	—	—	Y	—
28.	0·83	Y	—	Y	—	—	—	—	—
29.	0.86	—	—	—	—	—	—	—	GY*
30.	0·90	—	—	—	—	—	Y	Y	—
31.	0·94	—	—	—	—	—	GY	—	—
32.	0·97	—	—	—	—	—	—	—	Y
33.	1·00	BY	Br	BY	Br	Br	Br	Br	Br

Oberonia pattern : Numbers 1 to 8 indicate the species of *Oberonia* viz. : *O. falconeri*, *O. verticillata*, *O. ensiformis*, *O. brunoniana*, *O. santapaui*, *O. iridifolia* var. *denticulata*, *O. brachyphylla* and *O. proudlockii*,

Table 2 : Summary of flavonoid spot patterns in the species of genera *Malaxis* & *Liparis*

Spot No.	Rf value	Colour of spots in *Malaxis* pattern			Spot No.	Rf value	Colour of spots in *Liparis* pattern	
		1	2	3			1	2
1.	0·044	GY*	GY*	GY*	1.	0·033	Y	Y
2.	0·088	Y	Y	—	2.	0·23	—	Y
3.	0·11	—	—	Y	3.	0·33	Y	Y
4.	0·21	Y	Y	Y	4.	0·43	—	Y
5.	0·31	Y	Y	—	5.	0·50	Y	Y
6.	0·33	—	—	GY*	6.	0·60	GY*	GY*
7.	0·38	GY*	GY*	—	7.	0·60	—	BY*
8.	0·41	—	—	B*	8.	0·64	GY*	—
9.	0·47	—	B*	—	9.	0·68	B*	—
10.	0·50	B*	—	B*	10.	0·32	—	B*
11.	0·57	—	—	Y	11.	0·86	GY*	—
12.	0·60	—	Y	—	12.	0·88	—	Y
13.	0·64	Y	—	—	13.	0·97	R*	R*
14.	0·65	—	—	B*				
15.	0·77	GY*	GY*	B*				
16.	0·87	Y	Y	—				
17.	0·88	—	—	Y				
18.	0·98	Br	Br	YBr				

Malaxis pattern : Numbers 1 and 3 indicate species of *Malaxis*, viz. : *M. versicolor* and *M. densiflora* respectively. Numbers 2 indicates the cytotype (coll. No. 1626) or *M. versicolor*.

Liparis pattern : Numbers 1 and 2 indicate species of *Liparis* viz. : *L. nervosa* and *L. prazeri* respectively.

*= maker spots.

Table 3 : Summary of free amino acid pattern

Sl. No.	Rf Value	*Oberonia* species								*Malaxis* species		*Liparis* species	
		1	2	3	4	5	6	7	8	1	2	1	2
1.	0·02	—	—	—	—	—	—	—	—	P*	—	—	—
2.	0·07	—	—	—	—	—	—	—	—	—	P*	—	—
3.	0·08	BrR	P	—	—	—	—	—	—	—	—	—	—
4.	0·09	—	—	P	BP	P	P	—	—	—	—	—	—
5.	0·10	—	—	—	B	-	—	P	P	—	—	—	—
6.	0·11	—	—	—	ORN	ORN	ORN	—	—	—	ORN	ORN	—
7.	0·12	LYS	—	—	—	—	—	LYS	LYS	—	—	—	LYS
8.	0·13	—	—	—	—	—	—	—	—	—	—	—	P
9.	0·13	—	—	—	—	—	—	—	—	—	ARG	—	ARG
10.	0·14	P	—	—	—	—	P	—	—	P	—	P	—
11.	0·15	—	—	—	—	YBr*	—	—	—	—	—	—	—
12.	0·16	—	—	—	ASP*	—	—	—	—	—	—	—	—
13.	0·17	—	—	—	—	—	—	P	P	—	—	P	—
14.	0·18	—	—	—	—	—	—	—	—	—	P	—	—
15.	0·19	—	—	—	—	P	P	—	—	P	—	—	—
16.	0·195	P*	—	—	—	—	—	—	—	—	—	—	—
17.	0·21	—	P	—	P	—	—	BrR	BrR	—	P	—	P
18.	0·22	—	—	—	—	—	—	—	—	P	—	—	—
19.	0·225	P	—	—	—	—	—	—	—	—	—	P	P
20.	0·23	—	Br*	—	—	—	—	—	—	—	—	—	—
21.	0·24	—	—	P	—	P	BP	BP	BP	—	—	—	P
22.	0·25	—	—	P	—	—	—	—	—	B*	—	—	—
23.	0·26	—	—	—	—	—	—	—	—	—	—	SER	—
24.	0.27	—	—	—	—	—	—	—	—	—	—	—	P
25.	0.28	GLY	GLY	GLY	GLY	GLY	GLY	GLY	—	GLY	GLY	—	—
26.	0·29	—	—	—	—	—	—	—	—	—	P*	—	—
27.	0·31	—	—	—	—	—	—	P*	—	—	—	—	—
28.	0·32	P	—	—	—	—	—	—	—	—	P	P	—
29.	0·33	—	—	P*	—	—	—	—	—	—	—	—	—
30.	0·34	—	—	—	P*	—	—	—	—	—	—	—	—
31.	0·35	—	—	—	—	—	—	—	—	—	—	—	—
32.	0·36	—	THR	—	—	—	THR	—	THR	—	—	—	—
33.	0·37	—	—	—	—	—	—	—	—	—	—	P	—
34.	0·40	—	—	P	—	—	—	—	—	P	P	P	P
35.	0·41	P	—	—	—	—	—	—	—	—	—	—	—
36.	0·42	—	—	—	—	—	P	P	P	—	—	—	—
37.	0·45	—	P	—	—	P	—	—	—	—	—	—	—
38.	0·48	—	—	—	—	—	—	—	—	—	—	—	—
39.	0·53	ILEU	ILEU	ILEU	ILEU	ILEU	ILEU	ILEU	ILEU	ILEU	ILEU	ILEU	ILEU

Oberonia species : 1. *O. falconeri*, 2. *O. Verticillata*, 3. *O. ensiformis*, 4. *O. brunoniana*, 5. *O. santapaui*, 6. *O. iridifolia* var. *denticulata*, 7. *O. brachyphylla*, 8. *O. proudlockii*. *Malaxis* species ; 1. *M. versicolor*, 2. *M. densiflora*. *Liparis* species : 1. *L. nervosa*, 2. *L. prazeri*,

*= Uncommon amino acid.

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